FULL, UNEDTIED GEL FOR FIG. S1

Test vorbereitet: 3.4
Gel vorbereitet: 4
Test durchgeführt:

Datum: 6.3.18
Uhrzeit:

...ngabe:

IEF-PROTO

Lanes used

	Patient				Ergebnis	Auffälligkeiten
1.	KO (+)			L	+	
2.	KO (−)			S	0	
3.	Patient 7 T1			L	+	
4.	Patient 7 T1			S	0	
5.	Patient 7 T2			L	+	
6.	Patient 7 T2			S	0	
7.	Patient 2 T1			L	+	
8.	Patient 2 T1			S	0	
9.	Patient 2 T2			L	+	
10.	Patient 2 T2			S	0	
11.	Patient 3 T1			L	+	
12.	Patient 3 T1			S	0	
13.	Patient 3 T2			L	+	
14.	Patient 3 T2			S	0	
15.	Patient 1 T1			L	+	
16.	Patient 1 T1			S	0	
17.	Patient 1 T2			L	+	
18.	Patient 1 T2			S	0	
19.	Patient 6 T1			L	+	
20.	Patient 6 T1			S	0	
21.	Patient 6 T2			L	+	
22.	Patient 6 T2			S	0	
23.	Patient 10 T1			L	+	
24.	Patient 10 T1			S	0	
25.	Patient 10 T2			L	+	
26.	Patient 10 T2			S	0	
27.	Patient 8 T1			L	+	
28.	Patient 8 T1			S	0	
29.	Patient 8 T2			L	+	
30.	Patient 8 T2			S	0	

31	Hb Human Kontrolle	Bemerkung:

Formblatt IEF-Protokoll Stand 25.02.2010

Supplementary Materials:

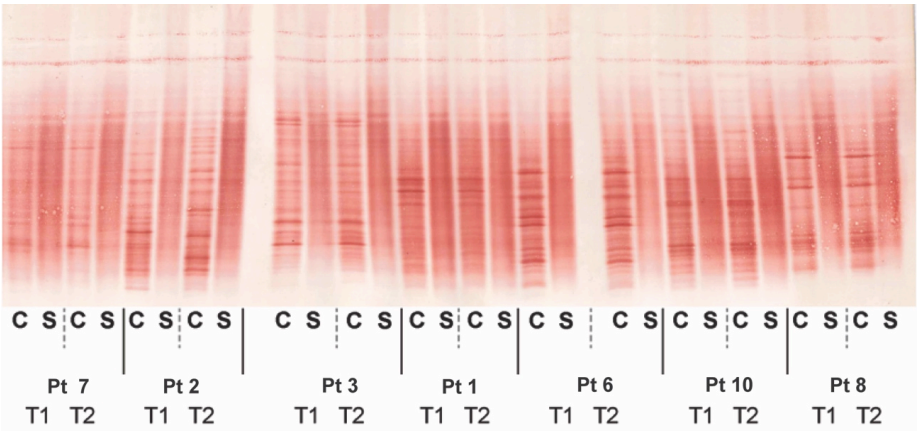


Figure S1. CSF-unique OCBs are mostly stable over time. Isoelectric focusing with IgG immunoblotting of CSF and serum at both time points for Pts 1-3, 6-8, and 10. The contrast and brightness of the original image were adjusted to improve visibility of bands, and the original image was flipped to present T1 and T2 in order and show CSF (C) before serum (S). The image was cropped to remove image parts that did not contain data relevant to this study. No specific features were enhanced, obscured, moved, removed, or introduced. The fact that patients do not appear in order is due to the order in which the respective samples were applied to the isoelectric focusing gel. All samples for a given patient were run the same gel. Pt, patient. CSF (C), cerebrospinal fluid. (S), serum. OCB, oligoclonal band. Pt, patient. IgG, immunoglobulin G. T1, time point 1. T2, time point 2. Refer to table S2 for description of CSF OCB comparisons between T1 and T2.

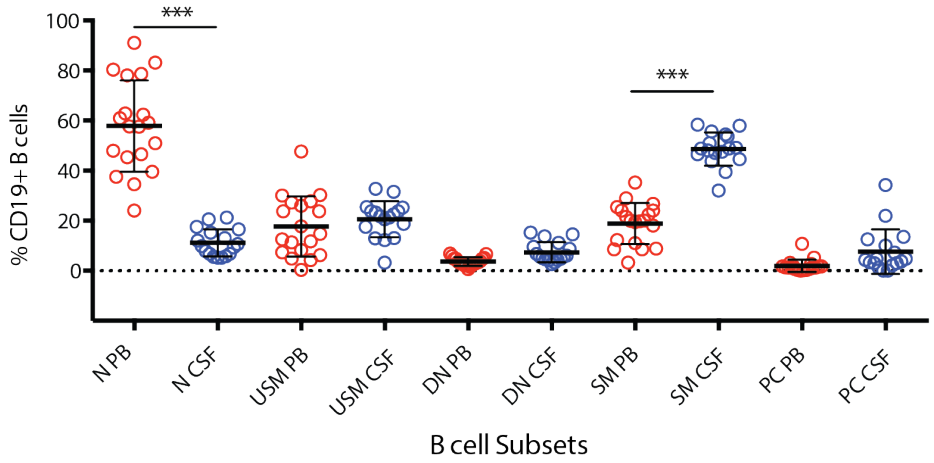


Figure S2: Naïve B-cells are more prevalent in blood; in CSF, SM B-cells are relatively increased. Shown are proportions of B-cell subsets in CSF (blue) and PB (red) among CD19+ B-cells as determined by multiparameter flow cytometry for N, USM, SM, DN, PC B-cell subsets. Overall CD19+ B-cells were 3.0% (+/- 3.0 SD) of all CSF lymphocytes and 7.2% (+/- 4.4 SD) of all PB lymphocytes. There were no significant differences between each subset per time point (not shown); therefore, shown here are combined data per subset from T1 and T2. T1-CSF subsets were measured in n=8 patients, in T2-CSF in n=9 patients, in T1-PB in n=9 patients, and in T2-PB in all 10 patients. Shown are naïve B-cells (N: CD19+IgD+CD27-), unswitched memory B-cells (USM: CD19+IgD+CD27+), class-switched memory B-cells (SM: CD19+IgD-CD27+), double negative B-cells (DN: CD19+IgD-CD27-), plasma cell (PC: CD27+CD38+ of CD19+IgD-), and CSF plasmablast/plasma cells (PC: CD19+IgD-CD27^{hi}). Comparisons between CSF and PB subsets were made using Anova (corrected for multiple comparisons using Sidak method); only significant differences are indicated, *** $p < 0.001$.

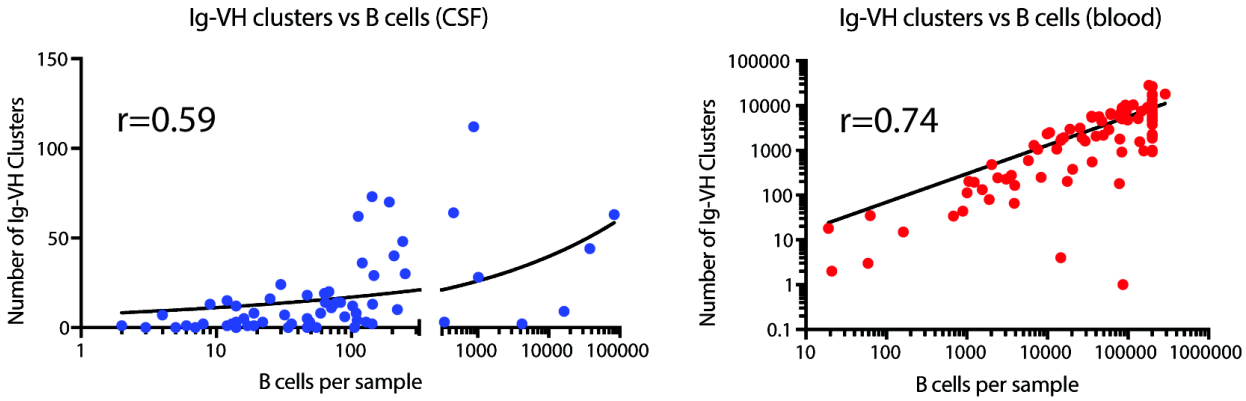


Figure S3. Number of Ig-VH clusters in a sample is correlated with cell count. Spearman correlation for number of Ig-VH clusters versus CSF B-cell count ($p<0.0001$) and for number of Ig-VH clusters versus PB B-cell count ($p<0.0001$) (log10 scale on x-axes as well as y-axis of PB plot). Ig-VH, immunoglobulin heavy chain variable region.

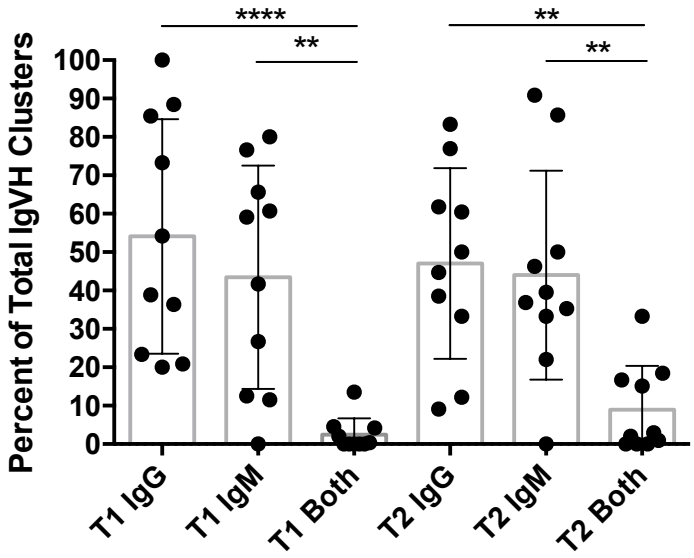


Figure S4. The majority of CSF immune repertoire Ig-VH clusters express either IgM or IgG. Each patient is represented by a point within each box plot showing the percentage of IgG-VH-only clusters or IgM-VH-only clusters, and of clusters with both IgG-VH and IgM-VH at T1 and T2. IgM, immunoglobulin M. Comparisons between Ig-VH cluster isotypes were made using Anova (corrected using Sidak method for multiple comparisons) in GraphPad Prism; only significant differences are indicated, ** $p < 0.01$, **** $p < 0.0001$.

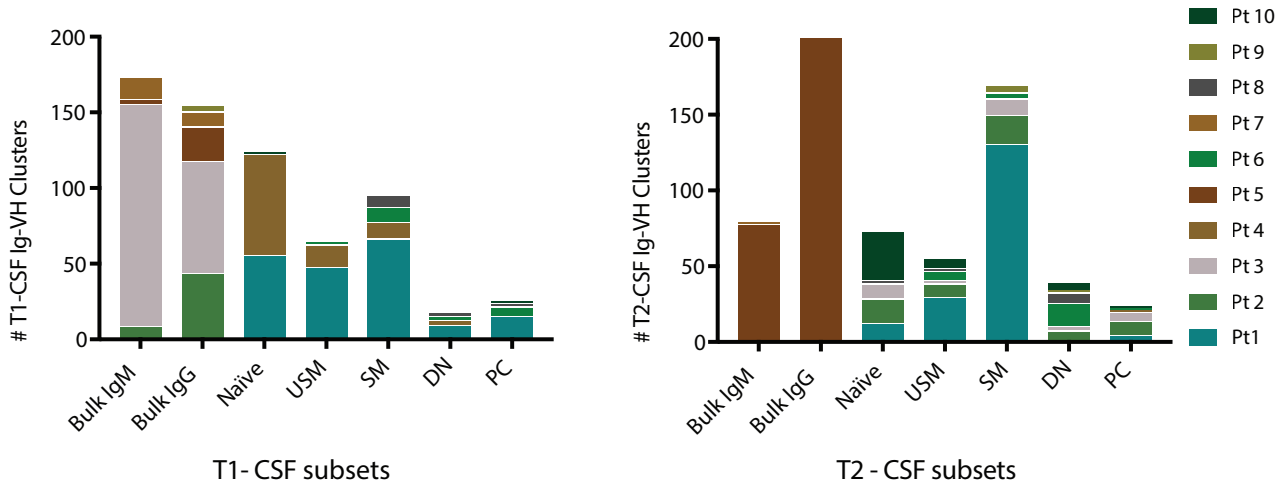


Figure S5. Different B-cell subsets compose CSF Ig-VH repertoires at T1 and T2. Of patients with sorted CSF B-cells, the number of Ig-VH clusters in T1-CSF and T2-CSF (and not in PB) containing each B-cell subset. As Ig-VH cluster is used as a unit of clonally-related populations in this study, this figure shows in which B-cell subsets these Ig-VH clusters have members. Bulk, unsorted B-cells.

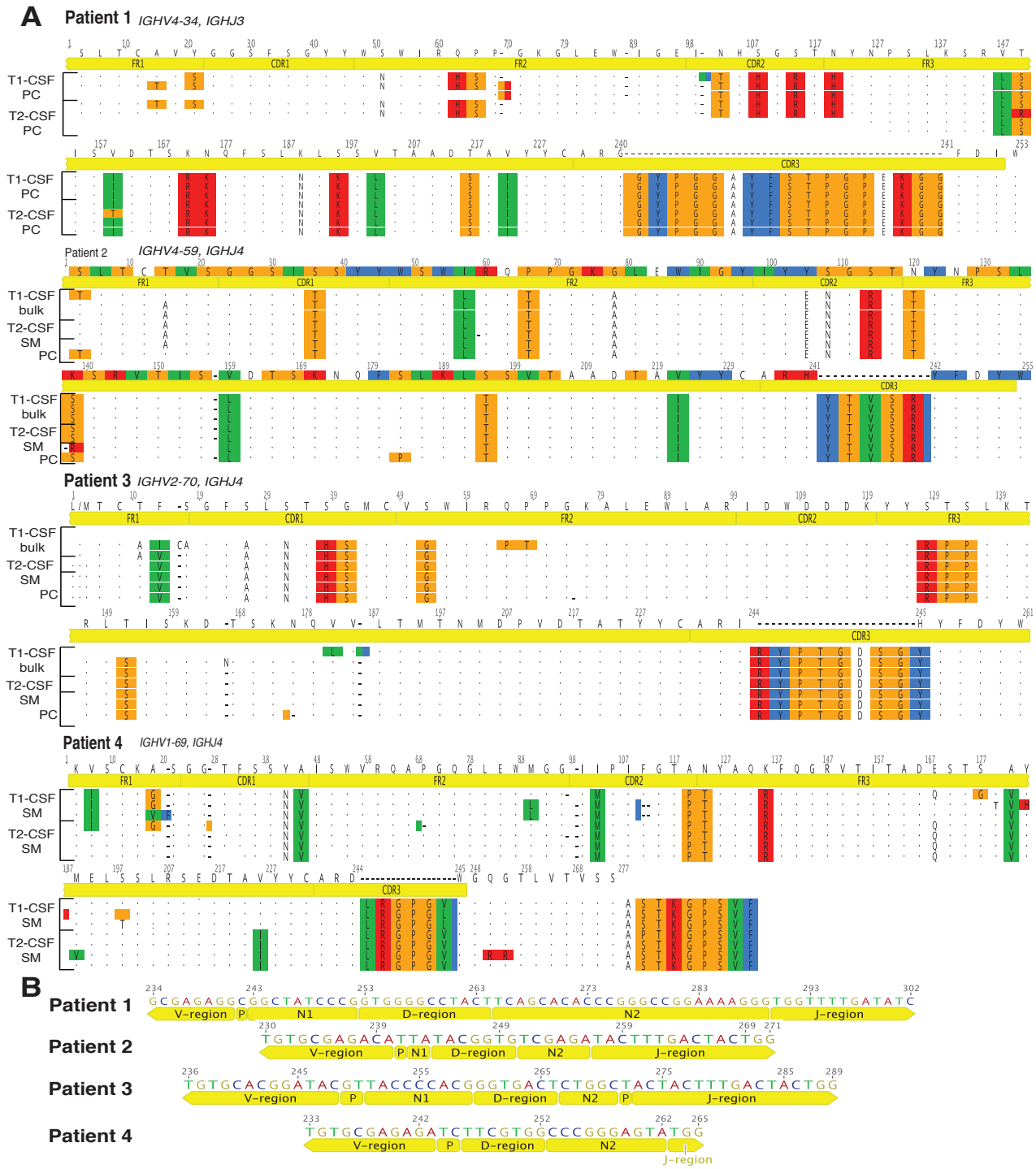


Figure S6. CSF-persistent Ig-VH clusters. A) Amino acid alignment of representative sequences from the indicated B-cell subsets from persistent Ig-VH clusters in patients 1-4 together with their related germline *IGHV* and *IGHJ*. Dots indicate amino acids identical to germline; color-shaded amino acids indicate differences from the germline. Regions of the immunoglobulin sequence are numbered and labeled according to IMGT (53) . Segments of the immunoglobulin sequence are labeled: FR1-FR3, framework regions 1-3; CDR1-CDR3, complementarity determining regions 1-3. B) Nucleotide sequence from CDR3 regions in the Ig-VH clusters depicted in (A). N and P nucleotide insertion sites at the V-D-J junctions forming the H-CDR3 are shown. Alignments generated using Geneious, IMGT V Quest(53) and NCBI IgBlast (54). *IGHV*, immunoglobulin heavy chain variable germline segment. *IGHJ*, immunoglobulin heavy chain joining germline segment.

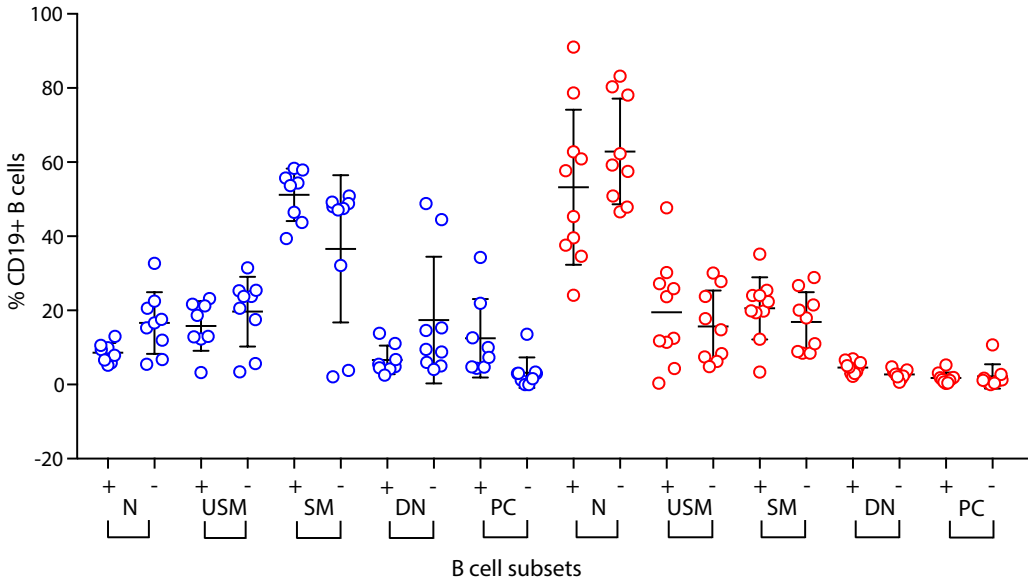


Figure S7. Patients with persistent CSF Ig-VH clusters show no significant difference in B-cell type prevalence in CSF or PB compared to patients without persistent CSF Ig-VH clusters. Blue circles: CSF. Red circles: PB. Shown are naïve B-cells (N: CD19+IgD+CD27-), unswitched memory B-cells (USM: CD19+IgD+CD27+), class-switched memory B-cells (SM: CD19+IgD-CD27+), double negative B-cells (DN: CD19+IgD-CD27-), plasma cell (PC: CD27+CD38+ of CD19+IgD-), and CSF plasmablast/plasma cells (PC: CD19+IgD-CD27^{hi}). (+), patients with persistent CSF Ig-VH clusters. (-), patients without persistent CSF Ig-VH clusters. T1-CSF subsets were measured in n=8 patients, in T2-CSF in n=9 patients, in T1-PB in n=9 patients, and in T2-PB in all 10 patients. Kruskal-Wallis with Dunn correction for multiple comparisons, $p<0.05$ was considered significant (none of the (+) vs (-) comparisons were statistically significant).

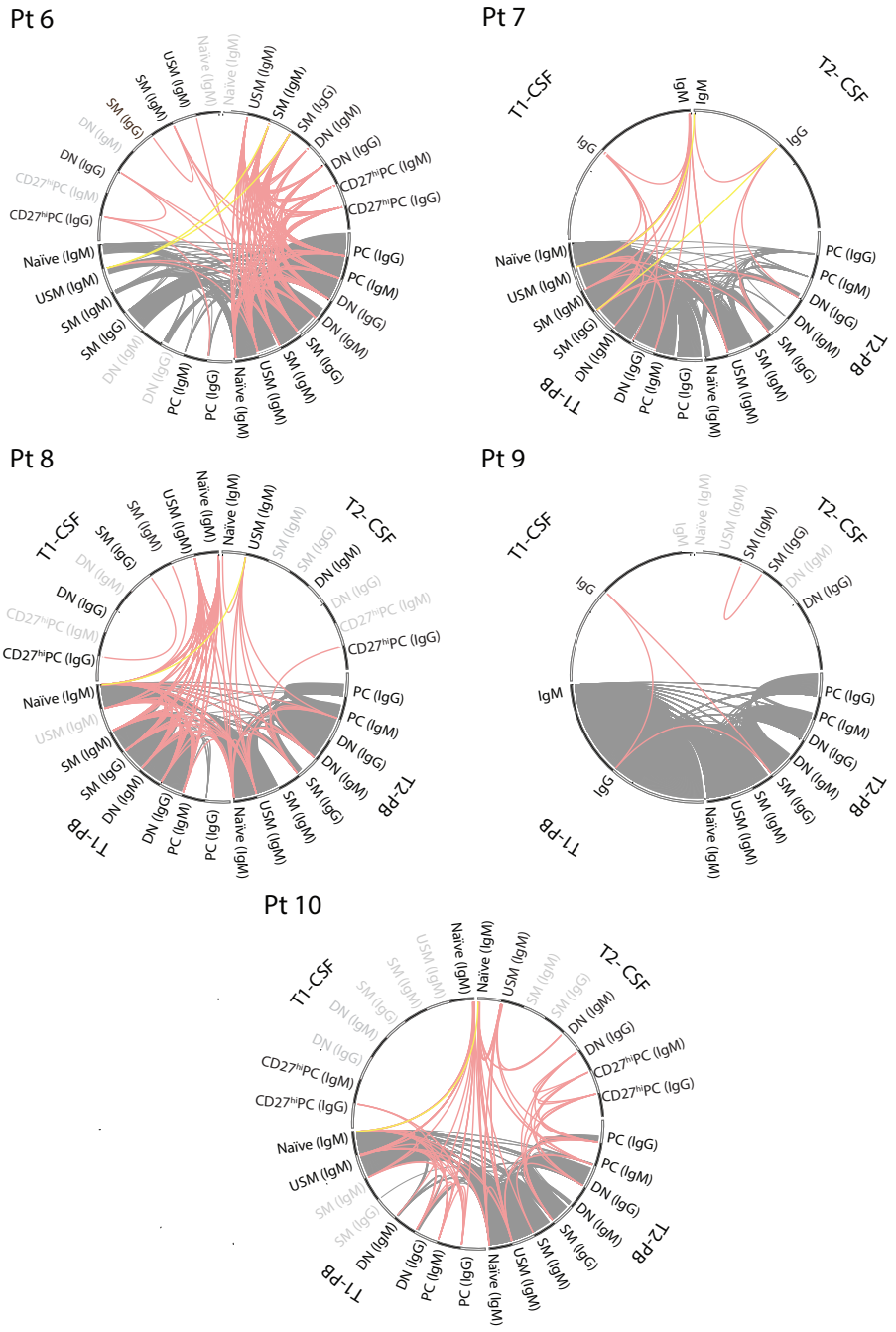


Figure S8. Patients without identifiable persistent CSF Ig-VH clusters still have clonal connections between CSF and PB. Within T1, Ig-VH clusters spanning CSF and PB are often SM, followed closely by USM. At T2, CSF-PB Ig-VH clusters are often SM. Clonal relationships between B-cell subsets are shown for each patient. Lines represent Ig-VH clusters shared between two subsets. Grey lines: PB-only Ig-VH clusters. Red lines: CSF-containing Ig-VH clusters. Yellow lines: T1-PB B-cell subsets, or bulk PB IgG and IgM (from Pt 9), that provide input to T2-CSF without involving T1-CSF. Grey font indicates subsets/Ig isotypes from which no Ig-VH libraries could be obtained.

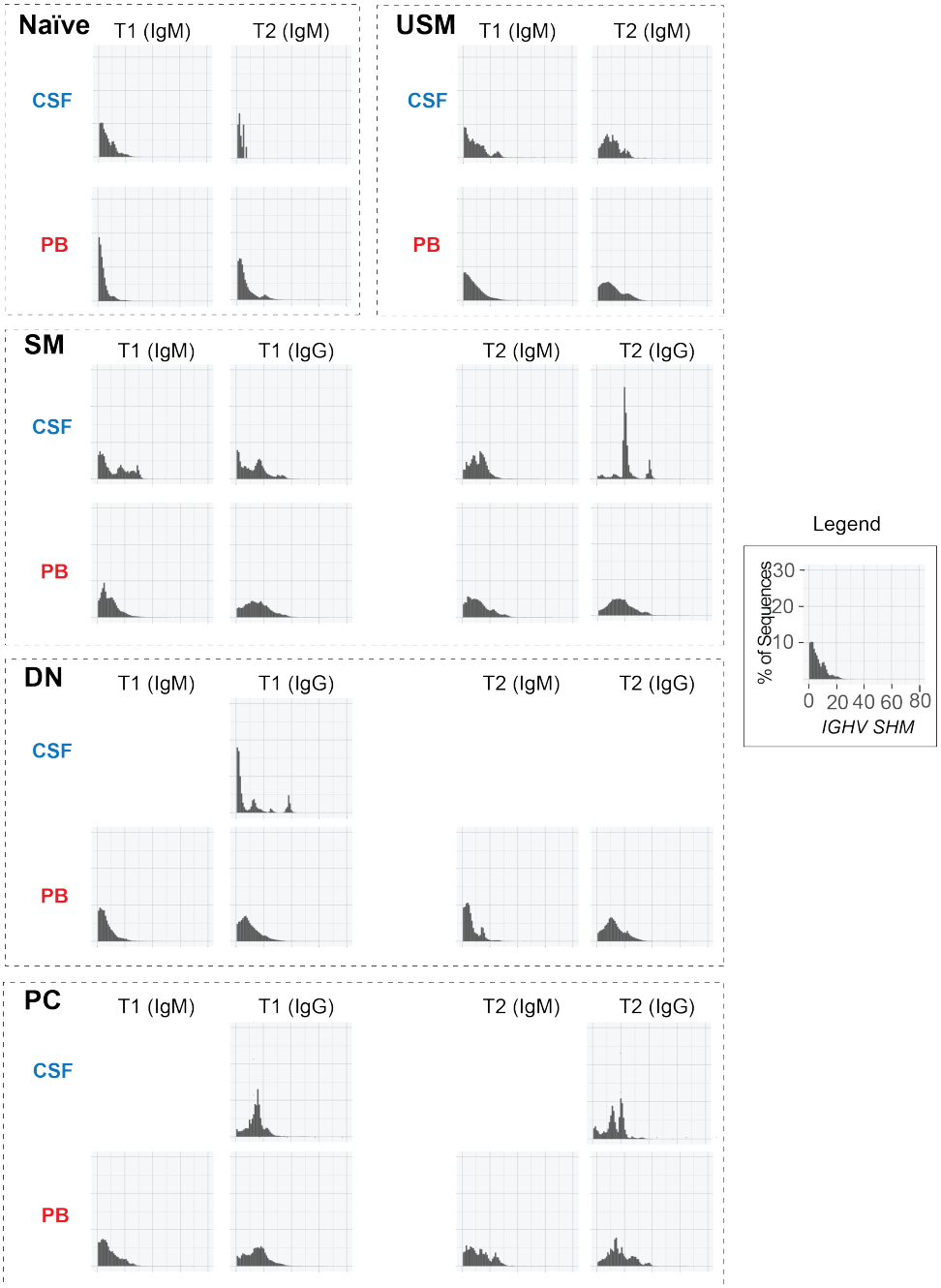
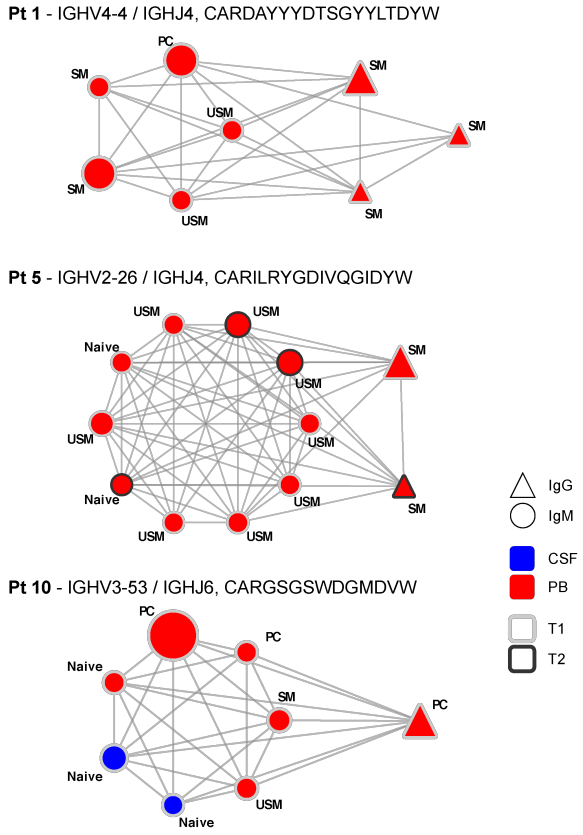


Figure S9. Somatic hypermutation rates follow expected patterns along B-cell lineage. Shown are somatic hypermutation profiles for B-cell subsets in CSF and PB from Patient 1. The x-axis shows the number of amino acid differences from reference germline *IGHV* sequences, i.e. mutations. The y-axis shows the percentage of sequences in the sample with a given number of mutations on the x-axis. Overall, the degree of somatic hypermutation follows the expected increase along the B-cell maturation stages as antigen exposure and affinity maturation occur: somatic hypermutation is least in naïve B-cells, and greater in IgG-expressing SM and PC. In this patient, there is a particularly high degree of SHM in IgG SM B-cells in T2-CSF. SHM, somatic hypermutation.



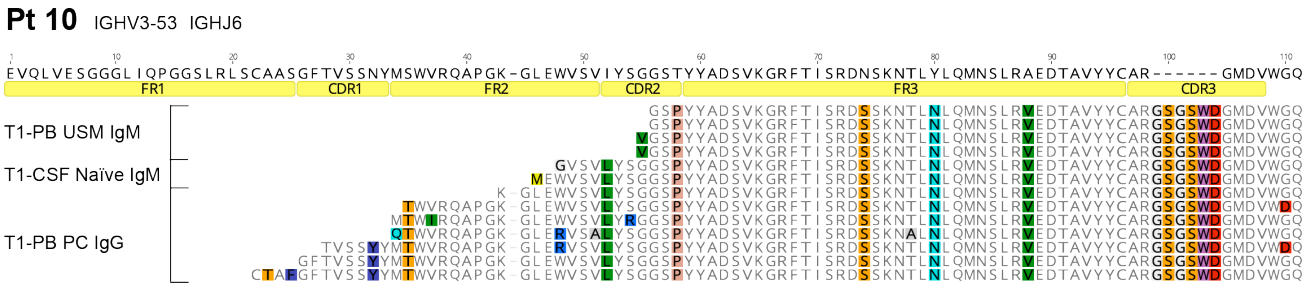


Figure S10: Clonal relationships between IgM-expressing USM B-cells and IgG-expressing B-cell subsets suggest Ig class-switch recombination and further maturation of USM B-cells. Shown are three representative Ig-VH cluster networks of clonally related B-cells, with *IGHV*, *IGHJ* and most common CDR3 amino acid sequences indicated per network. Each node represents a specific CDR3 expressed by the indicated B-cell subset; the node size is relative to the number of sequences found for each *IGHV*-*IGHJ*-H-CDR3 combination (range 2 to 317,112). IgM-expressing B-cell subsets are represented by circles, those expressing IgG by triangles. CSF B-cell subsets are indicated in blue, PB subsets in red; light gray rims indicate T1 subsets, dark gray rims indicated subsets that derive from T2. Shown below the cluster networks is an amino acid alignment of representative sequences from the indicated B-cell subsets from patient 10 together with the closest related germline *IGHV*. Color-shaded amino acids indicate differences from the germline. Regions of the immunoglobulin sequence are numbered and labeled according to IMGT (53).

Table S1. Patient characteristics based on presence or absence of CSF persistent Ig-VH

	Patients with persistent CSF Ig-VH clusters (n=5)	Patients without persistent CSF Ig-VH clusters (n=5)
Age (years)	30.4 (+/-3.1)	40 (+/-3.8)
Sex (M, F)*	0, 5	4, 1
Disease Duration (years)	2.2 (+/-1.2)	1.4 (+/-0.6)
EDSS	3.4 (+/-0.5)	2.7 (+/-0.8)
Time between T1 and T2 (months)	15.2 (+/- 1.8)	13 (+/- 1.1)
Clinical relapse between T1-T2	4	2
IgG Index (normal <0.66)	1.2 (+/-0.1)	0.9 (+/-0.1)
No. of Patients on IMT	4	3
Anti-lymphocyte trafficking IMT (i.e. fingolimod or natalizumab)	2	2
Gadolinium enhancement on MRI (at T1, T2)	4, 2	4, 2
CSF volume (mL)	11.5 (+/- 3.9)	13.7 (+/- 3.3)

Patients with and without persistent CSF Ig-VH clusters did not differ with respect to clinical metrics. More men had persistent CSF B-cells than women (*$p<0.05$ Fisher's exact test). EDSS, expanded disability status scale. IMT, immune modulating therapy. MRI, magnetic resonance imaging. IgG index, immunoglobulin G index ((CSF IgG/CSF albumin)/(serum IgG/serum albumin)).

Table S2. Clinical CSF biometrics.

Patient ID	Time Point	CSF WBC (cells/uL)	CSF collected (mL)	IgG Index (normal <0.66)	OCB	OCB Comparison
1	T1 T2	8 3	15 16	0.83 0.65	5 3	Decrease in number
2	T1 T2	12 10	6 10	1.1 1.75	>5 >5	Increase in number
3	T1 T2	7 3	7 17.5	NP 0.75	>5 >5	Stable
4	T1 T2	3 1	9.5 10	1.27 0.94	>5 >5	NP
5	T1 T2	4 4	14 10	1.64 1.55	>5 >5	NP
6	T1 T2	3 1	16 17	1 0.85	5 >5	Stable
7	T1 T2	2 1	9.5 11.5	0.9 0.74	>5 5	Stable
8	T1 T2	4 2	14 17	0.62 0.67	>5 >5	Stable overall: 1 band more prominent, 1 less prominent
9	T1 T2	0 0	9.5 13.5	1 0.62	>5 >5	NP
10	T1 T2	8 12	11 10	1.48 1.32	>5 >5	Stable overall: 1 band more prominent

Clinical diagnostic laboratory CSF WBC count, IgG index and OCBs present are shown for each patient at each time point. In n=7 patients, there was additional available CSF, and in these patients the pattern of CSF OCBs at T2 was compared to the OCB pattern at T1. NP, not performed. WBC, white blood cell. IgG index, immunoglobulin G index ((CSF IgG/CSF albumin)/(serum IgG/serum albumin)). OCB, oligoclonal band.

Table S3. B-cell samples analyzed by IgSeq.

Pt ID	Time Point	Sample Type	B-cell Subset	Number of B-cells	Isotype	Ig-VH Clusters	Exp	Raw Reads	Aligned Reads	
1	T1	CSF	Naïve	112	IgM	62	I	39948	20587	
			USM	142	IgM	73	I	49025	24978	
			SM	460	IgG	64	I	53241	23042	
					IgM	35	I	9615	5255	
			DN	83	IgG	14	I	37097	12231	
					IgM	0	I	0	0	
			PC	120	IgG	36	I	207795	86877	
					IgM	0	I	0	0	
		PB	Naïve	175179	IgM	9151	I	220760	41385	
			USM	84463	IgM	8832	I	339153	105179	
			SM	95720	IgG	7842	I	553275	180184	
					IgM	635	I	219349	103118	
			DN	19072	IgG	2963	I	626941	234604	
					IgM	99	I	73403	12421	
			PC	1055	IgG	202	I	846488	298704	
					IgM	124	I	195769	81500	
	T2	CSF	Naïve	77	IgM	14	I	659	0	
							TR	9456	1420	
							TR	162	0	
			USM	239	IgM	48	I	157	0	
							TR	26	0	
							TR	2989	1009	
			SM	880	IgG	112	I	142	67	
							TR	161	0	
							TR	5871	2323	
							TR	1332980	625014	
					IgM	83	I	59	0	
							TR	285201	159982	
							TR	192	13	
							TR	0	0	
			DN	47	IgG	0	I	339	0	
							TR	1325	0	*
					IgM	0	I	6480	0	
							TR	2143	0	
			PC	71	IgG	11	I	736	201	
							TR	330	0	
							TR	11873	4531	
					IgM	0	I	3	0	
							TR	0	0	
							TR	77	0	
		PB	Naïve	60917	IgM	6640	I	262400	49100	
			USM	79605	IgM	5480	I	266747	53849	
			SM	47200	IgG	4482	I	769897	220884	

					IgM	1101	I	231100	56482
			DN	14654	IgG	1675	I	609380	185738
					IgM	97	I	46488	12816
			PC	1229	IgG	194	I	550130	190036
					IgM	125	I	139235	50504
2	T1	CSF	bulk	37673	IgG	44	I	81628	45243
					IgM	7	I	1133	308
		PB	Naïve	200000	IgM	7822	I	152323	31911
			USM	146293	IgM	7450	I	162622	47212
			SM	200000	IgG	6793	I	423906	155698
					IgM	1117	I	81672	37788
			DN	43729	IgG	5654	I	577341	244324
					IgM	650	I	172128	95214
			PC	7585	IgG	1049	I	349470	149936
					IgM	211	I	50291	28296
	T2	CSF	Naïve	47	IgM	18	I	33126	17856
			USM	102	IgM	12	I	32198	19097
			SM	249	IgG	30	I	92267	32018
					IgM	11	I	2486	569
			DN	30	IgG	24	I	46805	19529
					IgM	0	I	34	0
			PC	68	IgG	20	I	50993	23903
					IgM	0	I	2151	1
		PB	Naïve	61533	IgM	6308	I	154660	39006
			USM	10634	IgM	2508	I	146168	57403
			SM	15881	IgG	1926	I	562184	184976
					IgM	285	I	83634	30364
			DN	6787	IgG	1297	I	337274	150492
					IgM	85	I	29104	15681
			PC	63	IgG	35	I	359362	114119
					IgM	8	I	107154	66322
3	T1	CSF	bulk	82900	IgG	63	I	1161091	522507
					IgM	140	I	209102	39341
		PB	Naïve	200000	IgM	9660	I	745241	161869
			USM	84400	IgM	5030	I	732578	209200
			SM	200000	IgG	925	I	1163560	373157
					IgM	365	I	352938	111239
			DN	170000	IgG	8572	I	538099	150224
					IgM	783	I	109361	25553
			PC	2410	IgG	245	I	985465	379640
					IgM	179	I	223450	100665
	T2	CSF	Naïve	14	IgM	12	I	635998	196072
			USM †	4	IgM	7	I	2083	172

			SM	63	IgG	19	I	875008	368535	
					IgM	0	I	291842	0	
			DN	17	IgG	1	I	1036	4	
					IgM	4	I	2311	114	
			PC †	9	IgG	13	I	666444	402357	
							TR	600838	492251	
					IgM	1	I	27352	1	
							TR	35400	3	
		PB	Naïve	200000	IgM	10223	I	461166	66739	
			USM	14878	IgM	1818	I	394218	124183	
			SM	133478	IgG	5088	I	830086	315358	
					IgM	459	I	114407	34347	
			DN	92953	IgG	10286	I	1548667	670757	
					IgM	854	I	287615	88721	
			PC	13038	IgG	1062	I	737288	330353	
					IgM	595	I	208666	62988	
4	T1	CSF	Naïve	190	IgM	70	I	33043	13483	
			USM	146	IgM	29	I	24853	8364	
			SM-PC	1022	IgG	28	I	53400	18138	
					IgM	0	I	0	0	
			DN	89	IgG	6	I	86651	12354	
					IgM	0	I	0	0	
		PB	Naïve	290198	IgM	18074	I	466054	97254	
			USM	89486	IgM	7123	I	415706	125276	
			SM	188264	IgG	8258	I	1160979	249186	
					IgM	1081	I	245366	78639	
			DN	35706	IgG	5492	I	1912127	469120	
					IgM	253	I	279055	60085	
			PC	1001	IgG	113	I	1008112	207739	
					IgM	50	I	89606	41889	
	T2	CSF	Naïve	19	IgM	1	I	16961	17	
							TR	2764	0	
			USM	34	IgM	0	I	83755	0	*
							TR	50290	0	
			SM	142	IgG	2	I	1863	1045	
							TR	18520	8543	
							TR	723	51	
					IgM	0	I	3	0	
							TR	2	0	
							TR	31	0	
			DN	14	IgG	0	I	622	0	
							TR	106	0	*
					IgM	0	I	4831	0	

							TR	764	0	
			PC	12	IgG	1	I	2266	559	
							TR	19903	10698	
							TR	675	0	
					IgM	1	I	510	0	
							TR	16334	11	
							TR	189	0	
		PB	Naïve	200000	IgM	2107	I	163845	8893	
			USM	200000	IgM	2133	I	140853	17597	
			SM	200000	IgG	9447	I	514188	138860	
					IgM	541	I	53707	6956	
			DN	40109	IgG	2082	I	584643	166918	
					IgM	474	I	81251	14395	
			PC	679	IgG	34	I	282182	92666	
					IgM	59	I	203153	51466	
5	T1	CSF	bulk	unknown	IgG	23	I	1361	325	
							TR	23091	7962	
							TR	571	0	
					IgM	3	I	132	0	
							TR	2595	386	
							TR	43	0	
		PB	Naïve	200000	IgM	4976	I	242268	49347	
			USM	200000	IgM	4523	I	257151	59188	
			SM	200000	IgG	1876	I	720826	249298	
					IgM	253	I	123144	46742	
			DN	81400	IgG	6827	I	746881	229740	
					IgM	246	I	35456	7616	
			PC	1890	IgG	80	I	223187	94706	
					IgM	147	I	595747	270258	
	T2	CSF	bulk	unknown	IgG	245	I	14221	2631	
							TR	1324791	564943	
					IgM	70	I	260	3	
							TR	14057	2843	
		PB	Naïve	200000	IgM	16802	I	366778	116156	
			USM	200000	IgM	4907	I	414710	202128	
			SM	200000	IgG	9098	I	896283	295044	
					IgM	472	I	50416	15033	
			DN	115849	IgG	10397	I	809315	258011	
					IgM	148	I	19478	3128	
			PC	162	IgG	15	I	543166	217384	
					IgM	20	I	178884	77425	
6	T1	CSF	Naïve	105	IgM	0	I	264476	0	*
			USM	128	IgM	3	I	327565	55744	

			SM	340	IgG	3	I	31953	14499	
					IgM	9	I	336665	158245	
			DN	110	IgG	4	I	325684	10319	
					IgM	0	I	32410	0	
			PC	19	IgG	8	I	764076	122329	
					IgM	0	I	8833	0	
		PB	Naïve	200000	IgM	1002	I	543933	5352	
			USM	78176	IgM	180	I	257389	36782	
			SM	156710	IgG	972	I	237251	13302	
					IgM	199	I	170953	14970	
			DN	35762	IgG	550	I	145676	3856	*
					IgM	16	I	7339	167	
			PC	889	IgG	44	I	394103	61993	
					IgM	28	I	185208	28922	
	T2	CSF	Naïve	45	IgM	0	I	0	0	*
			USM	64	IgM	14	I	396630	356366	
			SM	143	IgG	13	I	506718	391468	
					IgM	10	I	333972	260293	
			DN	25	IgG	16	I	688318	521557	
					IgM	5	I	112268	667	
			PC†	12	IgG	15	I	470279	378332	
					IgM	12	I	449574	349612	
		PB	Naïve	200000	IgM	17858	I	375931	182899	
			USM	78954	IgM	1792	I	453932	325066	
			SM	200000	IgG	5140	I	528853	365527	
					IgM	891	I	151177	99882	
			DN	98481	IgG	9108	I	668534	444711	
					IgM	412	I	116891	77454	
			PC	26941	IgG	1899	I	704335	489843	
					IgM	860	I	237060	171077	
7	T1	CSF	bulk	16399	IgG	9	I	50692	15008	
					IgM	15	I	762	324	
		PB	Naïve	200000	IgM	6026	I	152040	25046	
			USM	200000	IgM	4731	I	159363	39758	
			SM	200000	IgG	6839	I	437850	126749	
					IgM	2096	I	132495	48575	
			DN	86617	IgG	8579	I	429833	125093	
					IgM	1440	I	80089	23517	
			PC	5785	IgG	589	I	462064	166191	
					IgM	405	I	93627	44582	
	T2	CSF	bulk	4210	IgG	2	I	35623	18926	
					IgM	2	I	177	97	
		PB	Naïve	25417	IgM	3128	I	115211	45698	

			USM	9912	IgM	2335	I	193174	84241	
			SM	3078	IgG	228	I	946058	364072	
					IgM	54	I	190029	76307	
			DN	1551	IgG	132	I	421432	157055	
					IgM	4	I	13520	1396	
			PC †	19	IgG	18	I	143120	49583	
					IgM	10	I	531331	220280	
8	T1	CSF	Naïve	32	IgM	7	I	117980	76362	
			USM	59	IgM	8	I	121560	101569	
			SM	108	IgG	8	I	1627	867	
							TR	14353	5565	
							TR	380	0	
					IgM	1	I	31	0	
							TR	325	170	
							TR	6	0	
			DN	36	IgG	2	I	1412	234	
							TR	12793	4419	
							TR	375	0	
				36	IgM	0	I	84	0	
							TR	1378	0	
							TR	23	0	
			PC	8	IgG	2	I	1524	0	
							TR	290934	300	
							TR	14464	45	
							TR	430	0	
				8	IgM	0	I	1	0	
							TR	20	0	
							TR	334	0	
							TR	0	0	
		PB	Naïve	181753	IgM	28341	I	3938585	1131870	
			USM	14649	IgM	4	I	107	30	*
			SM	17624	IgG	203	I	969745	423212	
					IgM	131	I	249957	96678	
			DN	3909	IgG	165	I	758428	267570	
					IgM	75	I	145441	46026	
			PC	21	IgG	2	I	168262	105243	
					IgM	5	I	37121	27987	
	T2	CSF	Naïve	13	IgM	2	I	4724	1164	
			USM	22	IgM	3	I	8453	2550	
			SM	55	IgG	0	I	16	0	
					IgM	0	I	75	0	*
			DN †	7	IgG	0	I	4	0	
					IgM	15	I	9655	1999	

			PC	2	IgG	1	I	78520	32697	
					IgM	0	I	27699	0	
		PB	Naïve	200000	IgM	13265	I	551204	146660	
			USM	57800	IgM	2908	I	2020938	634198	
			SM	140118	IgG	1553	I	593767	182970	
					IgM	0	I	26	2	
			DN	20474	IgG	378	I	544174	160618	
					IgM	151	I	261438	87403	
			PC	3565	IgG	277	I	122810	38172	
					IgM	210	I	155272	55287	
9	T1	CSF	bulk	unknown	IgG	4	I	1960	874	
							TR	700	0	
							TR	18527	6855	
				unknown	IgM	0	I	59	0	
							TR	21	0	
							TR	1097	0	
		PB	bulk	unknown	IgG	960	I	1017427	703518	
					IgM	10559	I	3698830	2163377	
	T2	CSF	Naïve	3	IgM	0	I	25	0	*
			USM	5	IgM	0	I	20	0	*
			SM	16	IgG	5	I	17095	8150	
					IgM	1	I	34416	99	
			DN	6	IgG	1	I	9533	17	
					IgM	0	I	1031	0	
			PC	0	IgG	n/a	n/a	n/a	n/a	
					IgM	n/a	n/a	n/a	n/a	
		PB	Naïve	200000	IgM	26685	I	463680	172023	
			USM	35263	IgM	5757	I	440115	253640	
			SM	98748	IgG	4733	I	818738	452511	
					IgM	289	I	79508	39915	
			DN	29358	IgG	1621	I	767356	487204	
					IgM	186	I	73377	21868	
			PC	2031	IgG	484	I	494349	309270	
					IgM	106	I	42157	26743	
10	T1	CSF	Naïve	74	IgM	13	I	1494917	127665	
							TR	1088739	159791	
			USM	95	IgM	no PCR product				*
			SM	292	IgG					
					IgM					
			DN	96	IgG					
					IgM					
			PC	14	IgG	3	I	407278	189715	
							TR	474419	1018	

					IgM	1	I	60102	0	
							TR	72897	3	
		PB	Naïve	200000	IgM	16988	I	279121	60479	
							TR	351486	202860	
			USM	83694	IgM	916	I	226432	118098	
							TR	336683	223244	
			SM	86347	IgG	1	I	496	25	
							TR	1	0	*
					IgM	1	I	258700	59	
							TR	141	84	
			DN	8342	IgG	251	I	279017	176994	
							TR	264356	167374	
					IgM	38	I	20296	2961	
							TR	25569	5624	
			PC	59	IgG	3	I	314085	200898	
							TR	549128	443931	
					IgM	4	I	225174	142723	
							TR	423936	323116	
	T2	CSF	Naïve	206	IgM	40	I	1901177	105912	
			USM	218	IgM	10	I	531781	175800	
			SM	648	IgG	no PCR product				*
					IgM					
			DN	47	IgG	5	I	462534	140602	
					IgM	2	I	137253	62	
			PC	49	IgG	3	I	632600	188073	
					IgM	1	I	11956	16	
		PB	Naïve	200000	IgM	5779	I	339081	37038	
			USM	200000	IgM	3779	I	289896	66564	
			SM	200000	IgG	2280	I	621418	173182	
					IgM	323	I	187316	53827	
			DN	49382	IgG	2176	I	507080	123160	
					IgM	147	I	81149	9991	
			PC	3860	IgG	66	I	375733	131626	
					IgM	62	I	79833	30496	

On average 2118 (+/- 9953 SD) aligned reads were obtained per cell. Shown are the number of B-cells in each patient's sorted or bulk sample(s) at T1 and T2. Bulk samples contain all B-cells from a given time point sorted into a single sample tube; FACS-sorted B-cell subsets are naïve, USM, SM, DN, or PC. The number of IgG-VH/IgM-VH clusters derived from each sample's IgG-VH and IgM-VH sequencing libraries are shown as well as initial and technical replicate raw sequencing read counts and aligned post-MiXCR read counts. Exp, Experiment: I, initial. TR, technical replicate. FACS, fluorescence-activated cell sorting. *Subsets/Ig isotypes from which no Ig-VH libraries could be obtained. † 5 subsets yielded more Ig-VH clusters than the number of input cells. For these samples, we analyzed the most abundant Ig-VH clusters, such that the number of clusters did not exceed the number of input cells.

Table S4: CSF Ig-VH cluster persistence rate is similar to PB Ig-VH cluster persistence rate.

	% of T1 Ig-VH clusters (+/- SD)	**% of T2 Ig-VH clusters (+/- SD)**	**p-value** CSF-persistence vs PB-persistence (T1, T2)
CSF-persistence rate patients with persistent CSF Ig-VH clusters	5.4% (+/- 7.2)	13.1% (+/- 20.9)	n/a
PB-persistence rate patients with persistent CSF Ig-VH clusters	6.4% (+/- 2.9)	7.9% (+/- 3.2)	p=0.8 and p=0.6
PB-persistence rate patients without persistent CSF Ig-VH clusters	5.6% (+/-3.8)	4.3% (+/-5.5)	p=1.0 and p=0.5

Ig-VH cluster persistence rate is defined as the percent of total Ig-VH clusters from T1 or T2 that are found in both T1 and T2 samples (Persistence rate as % of T1 = # Ig-VH clusters found at both T1 and T2 / Total # Ig-VH clusters at T1). Persistence rate in the CSF is compared to the PB-persistence rate in the five patients with persistent CSF Ig-VH clusters as well as the PB-persistence rate in the five patients without persistent CSF Ig-VH clusters. Unpaired t-tests, $p<0.05$ was considered significant.

Table S5. FACS antibody sort panels.

Sort panel	**BV421**	**FITC**	**PerCP-Cy5.5**	**eF710**	**PE-Cy7**	**PE**	**APC**	**APC-Alexa750**
3	CD4	CD20	CD19	---	---	CD14	CD3	CD8
6	IgD	CD20	CD38	---	CD3	CD138	CD27	CD19
7	IgD	CD19	---	CD5	---	CD38	CD27	---
8	IgD	CXCR5	CD38	---	---	CD138	CD27	CD19
19	IgD	CXCR5	CD38	---	CD3	CD138	CD27	CD19
22	IgD	CD20	CD19	---	---	CD3	CD27	CD8
18	IgD	CXCR5	CD38	---	CD3	CD138	CD27	CD19

Panels of fluorescent antibodies used to identify and sort B-cell subpopulations by flow cytometry. IgD Brilliant Violet 421 (Biolegend 11-26c.2a), CD4 Brilliant Violet 421 (Biolegend OKT4), CD20 FITC (Beckman Coulter B9E9), CXCR5 FITC (Biolegend J252D4), CD19 FITC (Biolegend HIB19), CD38 PerCPCy5.5 (BioLegend HIT2), CD19 PC5.5 (Beckman Coulter J3-119), CD5 PerCP-eFluor710 (eBioscience YKIX322.3), CD3 PE-Cy7 (Beckman UCHT1), CD138 PE (Miltenyi 449), CD3 PE (Beckman Coulter UCHT1), CD38 PE (eBioscience 90), CD14 PE (eBioscience 61D3), CD27 APC (eBioscience O323), CD3 APC (Beckman UCHT1), CD19 APC-Alexa750 (Beckman J3-119), CD8 APC-Alexa750 (Beckman B9.11). BV421, brilliant violet 421.

Table S6. FACS antibody sort panels used on cerebrospinal fluid and peripheral blood.

Patient ID	Time point	PB sort panel (1)	PB sort panel (2)	CSF sort panel
1	T1	7		22
	T2	18		19
2	T1	7	3	
	T2	7	3	22
3	T1	7		
	T2	18		19
4	T1	7	3	22
	T2	7	3	6
5	T1	7	3	
	T2	7	3	
6	T1	7	3	8
	T2	18		19
7	T1	7	3	
	T2	7	3	
8	T1	7	3	8
	T2	18	3	19
9	T1			
	T2	18		19
10	T1	7	3	8
	T2	18		19

Panels of fluorescent flow cytometry antibodies that were used on each sample at each time point. See Table S5 for details of each sort panel.